

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as elected Office

Date of mailing (day/month/year) 26 February 2001 (26.02.01)	
International application No. PCT/US00/16816	Applicant's or agent's file reference CONND10.PCT
International filing date (day/month/year) 16 June 2000 (16.06.00)	Priority date (day/month/year) 18 June 1999 (18.06.99)
Applicant MITCHEN, Joel, R. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
16 January 2001 (16.01.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

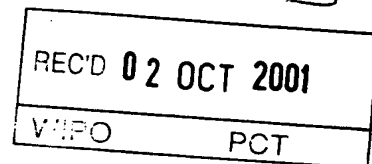
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer <p style="text-align: center;">R. E. Stoffel</p> Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference CONND10.PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US00/16816	International filing date (day/month/year) 16 JUNE 2000	Priority date (day/month/year) 18 JUNE 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant POLYMER TECHNOLOGY SYSTEMS, INC.		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of <u>4</u> sheets. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of _____ sheets.
3.	This report contains indications relating to the following items: <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of report with regard to novelty, inventive step or industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application

Date of submission of the demand 16 JANUARY 2001	Date of completion of this report 17 SEPTEMBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer LOUISE LEARY
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US00/16816

I. Basis of the report**1. With regard to the elements of the international application: ***

- ☒ the international application as originally filed
- ☒ the description:
pages 1-30 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____
- ☒ the claims:
pages 31-32 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____
- ☒ the drawings:
pages 1-5 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____
- ☒ the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
- ☒ the claims, Nos. NONE
- ☒ the drawings, sheets/fig NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

**Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US00/16816

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. statement**

Novelty (N)	Claims	<u>NONE</u>	YES
	Claims	<u>1-6</u>	NO
Inventive Step (IS)	Claims	<u>NONE</u>	YES
	Claims	<u>1-6</u>	NO
Industrial Applicability (IA)	Claims	<u>1-6</u>	YES
	Claims	<u>NONE</u>	NO

2. citations and explanations (Rule 70.7)

Claims 1-6 lacks novelty under PCT Article 33(2) as being anticipated by Miki et al.

Miki et al disclose a process for measuring cholesterol in low density lipoproteins present in a sample by optically measuring a reaction product of the living sample with a reagent. Miki et al disclose that the sample was reacted in the presence of a non-ionic surfactant and at least one member selected from a cyclodextrin and derivatives thereof. Miki et al also disclose measuring LDL-cholesterol in a living sample comprising reacting the sample with a cholesterol enzyme in the presence of cyclodextrin or derivative thereof. Further, Miki et al disclose a method for measuring LDL-cholesterol in a living sample in the presence of amphoteric surfactant and cyclodextrin and/or cyclodextrin derivatives. See the abstract, column 2, lines 15-68; column 3, lines 1-16; and column 5, lines 35-65.

Claims 1-6 lacks novelty under PCT Article 33(2) as being anticipated by Futatsugi et al.

Futatsugi et al disclose a method for measuring an amount of LDL-cholesterol in samples by optically measuring the reaction product. Futatsugi et al also disclose reacting the sample with an amphoteric surfactant and cyclodextrin and/or cyclodextrin derivatives. See column 3, lines 6-58 and column 9, lines 9-29.

Claims 1-6 lacks novelty under PCT Article 33(2) as being anticipated by Miyauchi et al.

Miyauchi et al disclose a method for measuring an amount of LDL-cholesterol in samples by optically measuring the reaction product. Miyauchi et al also disclose reacting the sample with an amphoteric surfactant and cyclodextrin and/or cyclodextrin derivatives. Note the abstract, column 1, lines 5-10 and column 6, lines 12-68.

(Continued on Supplemental Sheet.)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US00/16816

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12Q 1/44, 1/60, 1/26, 1/28, 1/00; C08B 37/16 and US Cl.: 435/19, 11, 25, 28, 5; 536/103.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Claims 1-6 meet industrial applicability under PCT Article 33(4).

----- NEW CITATIONS -----

NONE

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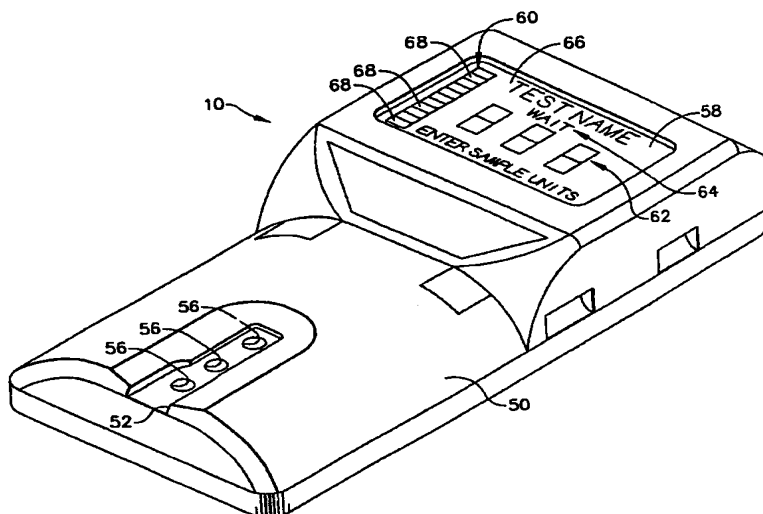
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ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: APPARATUS AND METHOD FOR DETERMINING SUBSTANCES CONTAINED IN A BODY FLUID



(57) Abstract: The invention describes methods for determining cholesterol in low density lipoproteins (LDL) in a living sample by reacting the sample with a reagent in the presence of an non-ionic surfactant and at least one member selected from the group consisting of cyclodextrin and derivatives thereof using novel techniques. An apparatus (10) for the optoelectric evaluation of test paper strips (12) for use in the methods for detection of certain analytes in blood or other body fluids is also provided. The figure shows a reflectance photometer (10) used to perform the methods of this invention and includes various features, including a lot number reader (66) wherein if the test strip (12) does not match the memory module (84), a test is not performed, and the user is instructed to insert a correct memory module (84).

APPARATUS AND METHOD
FOR DETERMINING SUBSTANCES
CONTAINED IN A BODY FLUID

5 BACKGROUND OF THE INVENTION

The present invention relates generally to an assay system for biological and nonbiological fluids. More particularly, the present invention relates to an apparatus for separating serum or plasma in order to measure analytes within the
10 serum.

It has long been desirable to utilize devices that can be used for on-site testing of blood products. Particularly important is the analysis of body fluids from humans and animals to diagnose disease, monitor the course of therapy, or determine the presence of illicit drugs. Human Biological fluids may include
15 blood, urine, saliva, feces, peritoneal fluid, synovial fluid, spinal fluid, interstitial fluid, tears, cellular fluid, pancreatic or bile fluids. Commonly, the analytical methods used to carry out these objects are performed on blood samples.

Clinical chemists have a preference for working with serum over plasma and plasma over whole blood because of the clarity of the sample matrix and the
20 lack of interfering substances from the solid portion of the blood. In order to facilitate analysis, a separation step must be carried out since the presence of red blood cells, either intact or hemolyzed interferes with detection of the signal generated by the chemical reaction performed by the test.

Conventionally, the separation of blood components has been carried out
25 by placing a clotted blood sample in a centrifuge and centrifuging the sample for

ten minutes at approximately 3,000 rpms. The serum obtained from this centrifuging step is then used to carry out the test, thus avoiding interference from blood solids such as red blood cells and clotting factors.

An embodiment for chemical tests called dry reagent strips was developed first for urinalysis. Thereafter, various efforts to combine dry reagent strip technology in blood testing were started in the early 1950's. Notably, U.S. Patent No. 3,092,465 discloses a reagent in a bibulous carrier with a superimposed semipermeable coating to exclude the chemical and nonchemical interference from red blood cells. The device, while performing analysis on whole blood, still required additional manipulations by the user, in the form of washing of excess blood after a specified time interval. Additionally, U.S. Patent Nos. 3,552,925 and 3,552,928 disclose the use of salts and amino acids to perform in-situ separation. U.S. Patent No. 4,477,575 discloses the use of a glass fiber matrix.

More recently, membranes have been employed in a variety of devices. These include devices disclosed in the following United States and foreign patents and publications: U.S. Patent Nos. 4,774,192 and 5,166,051; European Published Applications EP 0408222 A1, EP 0408223 A1, EP 0407800 A2 and EP 0388782; and PCT Published Applications Nos. WO 93/22453, WO 95/16207 and WO 90/10869. The use of the various membranes disclosed in the above patent documents operate on size exclusion principles, and several of these are limited by rates of capillary flow and do not completely eliminate interference from intact or hemolyzed red blood cells. Fresh red blood cells are elastic in nature and may pass through pores smaller than their nominal diameter. Hemolysis may occur on contact with some of the architectural or chemical components of

the strips. Consequently, errors may be introduced into the measurement system.

U.S. Patent No. 5,104,619 discloses a disposable diagnostic system comprising a test card having a substantially flat body and a generally cylindrical reagent pad pocket formed in a central area of the flat body. A reagent chemistry pad is disposed in the pocket and a snap fit cover is received in the pocket and arranged over the pad to retain the pad in position. The device size and configuration allows for bar code graphics to be printed on the underneath side of the device. The bar code may contain lot-specified data about the reagent chemistry, and is read by the meter during device insertion. This data may further contain critical parameters for the software algorithm within the meter electronics. U.S. Patent No. 5,139,685 also discloses a separation filter assembly having a snap fit lid. In this patent, glass fibers are utilized and maintained in a compressed state under pressure.

Accordingly, a need exists for an integrated system for assaying, in one step, analytes in whole blood samples which are not affected by the chemical or physical interference normally caused by red blood cells and other portions of whole blood.

20

SUMMARY OF THE INVENTION

The present invention, in one form thereof, comprises a dry solid phase diagnostic test strip and system for the chemical, enzymatic, and/or immunological analysis of whole blood analytes, comprising a reflectance photometer, a solid support strip, a porous detection zone member, a permeable

spreading and separation layer, a precipitation layer and an overlay sample receiving membrane containing an agent, or agents for the exclusion of intact red blood cells and a strip receiving platform with guides for positioning the strip inside the reflectance photometer. The detection area membrane may contain

5 chemical, enzymatic, and/or immunological reagents that generate specific signals in the presence of a target analyte. The agent, or agents, in contact with the overlay membrane, prevents passage and hemolysis of red blood cells while facilitating rapid transport and reaction of the plasma or serum portion of introduced whole blood samples. This method can be used to assay nearly every

10 component in whole blood. For example, the lipid fractions of the blood can be individually measured. A good method was a common reagent membrane for total cholesterol determination in combination with separation layers specific to the particular fraction of interest.

15 In addition, the present invention, in one form thereof, comprises a reflectance photometer, which utilizes test strips that are color coded for test differentiation, thereby making complicated tests less prone to human error. For example, a blue strip may indicate a glucose test, whereas a red strip may indicate a cholesterol test. These colors are then divided into shades such as

20 shades of blue equal to 64 lot numbers of glucose strips. The photometer includes a separate optical read head that determines the color and shade of the base of the test strip device as the strip is inserted into the photometric instrument. The shade is converted into a lot number ranging from 1 to 64. The instrument also has a memory module (preferably an electrically erasable

25 programmable read-only memory) that has a corresponding lot number to the

shade of the strip to ensure lot number verification. The instrument then compares the inserted memory module programmed lot number to ensure that it is the same lot number as the test strip. If the strip lot number does not match the memory module lot number, the test is not performed, and the user is

5 instructed to insert the correct memory module.

The lot number verification allows for the automated coding of lot numbers so that the user does not need to enter a lot code for each vial of strips. This prevents the running of the incorrect, old, or expired lot number tests in the instrument.

10 The "plug-in-memory" of the module includes the lot number of the test strip, the expiration date, and the performance criteria for the actual strip measurement. The performance criteria include the wavelength, measurement algorithm, and unreacted density qualifications necessary for a valid test result.

The optoelectronic measurements of the chemistry test reaction on and in

15 a surface enhances the dynamic range of the dry phase test. Algorithms that read at different wavelengths at different times in the chemistry reaction can extend the dynamic range of the test system. This is particularly applicable when using multiple chromophores in a single measurement system. The early portion of a chemistry could be read at the peak wavelength of a reaction, while the later

20 portion, or darker or more dense portion of color development could be read at a wavelength not near the peak of the color development. In addition, different chromophores may respond in a more linear manner in different portions of the dynamic range of the chemistry. Manipulation of these two data points can significantly increase the dynamic range (in mg/dl) of a chemistry reaction.

The optoelectronic measurement of the chemistry test reaction on and in a surface reduces errors due to orientation of the surface to be read to the instrument. Multiple wavelengths and different angles are used to correct possible problems in positioning the strip in the instrument. If the detector is at 5 "0" angle and the emitters of the same or different wavelengths are at different angles (e.g., one at 40° and one at 50°), the tilting of a surface will positively contribute to one reading while it will contribute in a negative manner to the other reading, thus it is able to cancel the error presented by the angle presentation of the surface. These same measurement methods can be used to eliminate 10 interferences from substances such as bilirubin and others.

The optoelectronic measurements of the chemistry test reaction on and in the surface enhance the stability of timed and untimed dry phase chemistry reaction. Algorithms are used to determine the "end point" of a chemistry. In other words, measurements can be done at similar or dissimilar wavelengths to 15 predict the stable portion or end point of a chemistry if kinetic measurements are made, the kinetic readings can be subjected to an algorithm to determine that the rate is slow enough to declare the extrapolate chemistry is at an end or completion. When known standards are run and predicted by this pseudo-endpoint, the same measuring criteria can be applied to unknowns to determine 20 the "endpoint" of the test reaction.

The use of colored or shaded visual indicators in the instrument enhance the interpretation of test results. A colored bar graph is used to aid the user in knowing when the user test results are in a normal or safe range. Out of range colors such as orange for caution and red for danger are used when results are 25 outside the green "safe" range. This is particularly useful to new testers who are

not familiar with the number scale of the different test results. A voice module can also be used to warn the user of unsafe results or operation of the instrument system to make the system usable by the visually impaired by providing, for example, a sound beep for each unit of glucose during a glucose test.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of the reflectance photometer in accordance with an embodiment of the present invention;

Fig 2 is an exploded perspective view of the plastic test strip of present invention
10 in its unlocked position;

Fig 3 is a perspective view of the plastic strip of Fig. 2 in its locked position; and

Fig 4 is a sectional view of the plastic strip;

Fig. 5 is a block diagram schematic of one embodiment of the reflectance photometer of the present invention;

15 Fig. 6 is a graph plotting sample size, elapsed test time and percentage of reflectance illustrating how endpoint determinations may be utilized to speed chemistry measurement.

BRIEF DESCRIPTION OF THE PREFERRED EMBODIMENT

20 In accordance with the embodiment of the present invention, the diagnostic chemistry measurement device 10 for dry solid phase chemical, enzymatic, immunological assay of whole blood or sera analytes is made up of an injection molded carrier test strip 20 in which several porous and nonporous materials containing chemicals and reactants are contained for the purpose of
25 generating a detectable signal in the presence of certain analytes. The test strip

12 is inserted into a reflectance photometer. The reaction material layer and the test strip 12 is held in intimate noncompressed contact with a whole blood separation layer in the absence of adhesives for the purpose of providing a liquid sample free of red blood cells to the reaction layer or layers.

5

HOLDER

The holder test strip 12 of this invention acts as holder for the different layers of the test reaction system. It provides a convenient handle as well as a mechanism for placing test strip 12 into an instrument 10 for the reading of the density changes of the reaction layers. As shown in Fig. 2 test strip 12 includes an elongate body 16 preferably formed by injection molding. Elongated body 16 includes a first end portion 18 and a second end portion 20. A hinged portion 22 is located between first and second end portions 18 and 20 so that first end 18 is foldable over elongated body 16 into contact with second end 20.

15 As shown in Fig. 2 first end portion 18 includes an opening 24 while second end portion 20 includes a complementary spaced opening 26. When first end portion 18 is folded over body 16, each opening 24 and 26 are aligned. In its folded position as shown in Fig. 3 opening 24 in test strip 12 defines an area for depositing a body fluid sample while opening 26 defines an area in which
20 optoelectronic measurements of chemistry test reactions are conducted.

Test strip 12 further includes a non-adhesive carrier layer 14 formed from, for example, five particular layers. In a standard diagnostic test strip, carrier layer 14 may include a disbursement layer 28, formed of, for example, woven materials such as polyester or cotton, for rapid and even disbursement of body fluid along
25 carrier layer 14. Beneath that may be included a separating binding and/or

precipitating layer 30, constructed of known materials such as shown in Table IX infra, that, when exposed to a sample liquid, may separate analyte and analyte disrupting elements such as red blood cells from whole blood. This action would permit the serum analytes to pass through separating layer 30a and 30b while
5 preventing red blood cells or other analyte disrupting elements from passing through. The last layer shown in Fig. 2 is that of the test reaction membrane 32 on which the dry chemicals and reactants are contained for generating a visible signal, in the presence serum analytes. Molded carrier body 16 serves as a support for the reacting and non-reacting layers 28, 30 and 32 which may be
10 formed from papers, membranes and other materials.

The test strip holder 12 positions the different layer materials 28, 31, 32 within the holder the correct X, Y and Z axis positions. Carrier layer 14 made up, for example, the disbursement separating and test reaction layers 28, 30 and 32 are held in noncompressed non-adhesive locations by first end portion 18 folding
15 over to second end portion 20. This may be accomplished in a number of different ways. The preferred way of noncompressingly holding carrier layer is of an upstanding annular rim 34 may help locate the carrier layer 14 within test strip 12. Additionally, small upstanding protuberances 36 along second end portion 20 radially located away from opening 26 prevent movement of carrier layer 14. The
20 purpose of both annular rim 34 and small upstanding protuberances 36 is to hold the layers of carrier layer 14 without compression between opening 24 and opening 26, hereby preventing pooling of any sample within carrier layer 14. This consideration of noncompression of the carrier layer 14 is of greater importance when larger numbers of layers are utilized. The positioning of a carrier layer 14
25 without adhesives or compression allows for efficient transport of sample and

reactants contained in the system and test strip 12. Annular rim 34 or alternatively other areas of test strip 12 may include sawtooth protrusions to increase flow rate thorough carrier layer 14.

Test strip 12 includes a locking mechanism to prevent any unlocking of front-end portion 18 from its folded position over elongated body 16. As shown in Fig. 2, one type of locking mechanism may include a plurality of upwardly extending tabs or projections 38 that interfit or lock into corresponding openings 40 in first end portion 18. When first end portion 18 is folded to second end portion 20, lock projections 38 will interfit and snap lock with openings 40. Other types of one way locking mechanisms may also be used such as snap rivets.

More than one test reaction system can be housed in a test strip 12. A second set of holes 24, and 26 may be included in test strip 12 so that two tests may be run at once.

The described holding mechanism allows for the rapid separation of whole blood into its liquid and solid components. It also allows sample volumes as low as 2.0 microliters to be used in dry phase chemistry reactions. Test strip 12 allows the use of several reaction and non-reaction layers. A typical holder could contain from 1 to 8 layers of material with thicknesses from approximately 0.002 inches to 0.007 inches, for example.

Chemicals and materials are employed to allow for the treatment of samples such as whole blood, which will allow the whole blood sample to be separated without disrupting the red blood cells while rapidly moving the liquid portion of the whole blood sample to one or more reaction sites in the holder, normally on a test reaction membrane 32. These chemicals can be composed of polymeric and nonpolymeric substances that are dried onto one or more surfaces of the

materials contained in the device holder. Additionally, light metal salts of elements such as Potassium, Lithium, Sodium, and Calcium may be utilized to treat red blood cells before and during the separation process. The materials which may be used in the holder for treatment by or containment of these
5 chemicals can be composed of woven, nonwoven, napped or flocked materials.

LDL

The present invention includes a homogeneous dry phase assay for LDL Cholesterol that can replace assays that previously required more than one
10 operation to achieve the desired measurement. Enzymes are used to digest HDL Cholesterol. This method uses a chemical complexing layer (ex. Surfynol 465, Surfynol 1485, Silwet L 7600, nDodecyl-B-D maltoside, Polyoxyethylene ether, Sucrosonomocaphate. A hydrophobic barrier does not allow the complex formed in #2 to react with the cholesterol reaction membrane. The best example of this
15 is a Polyether sulphane asymmetric, treated with casein, to make it hydrophobic. Casein coated membrane will not function alone because of its hydrophobicity: this property is adjusted by the addition of sorbitol and other agents, such as sucrose or tween 20. In comparison to PCT #WO95/16207, a barrier to control flow through the device is used, in contrast to the devices described in this
20 publication. This device does not have an absorbent or wick at far end of device, and this is not a chromatographic device. It was found that when the flow of liquid into the capillaries of a dry device encounters a restrictive barrier, the change in pressure due to this resistance can accelerate in one direction only until the device (the final reaction membrane) is wetted by the sample.

Thus, the sample flows in one direction in a more rapid rate than any other direction. This unidirectional flow allows for the chromogen and other reactants to move in one direction only without the use of an absorbent as in W095/16207.

Thus, the flow rate is controlled by the use of multiple holes, dissimilar in size,

- 5 (not unlike adjusting a hose sprayer nozzle.) Examples include: aperture sizes for aperture A, 0.050-0.250 and aperture B, 0.2-2.0 microns, and for Aperture C, whatever test strip holder size is. The apertures control unidirectional flow without using a proximal (final) absorbent layer (wick). Other devices with apertures contain a wick downstream. This construct is also used for HDL.

10

HDL

- The present invention includes a single reaction membrane with low holdup volume for the precipitation and/or binding of VLDL and LDL. This makes it possible to rapidly detect HDL in one step using whole blood. This direct flow-
- 15 through device is different than devices from BMC, Kingston and Cholestech that are all tangential flow systems.

Use of CHAPS in place of cholate in cholesterol reagent

- CHAPS, or 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate, is
- 20 made by Research Organics of Cleveland, Ohio, for use in cholesterol reagents because of problems people were having with cholate, the usual surfactant in diagnostic cholesterol reagents. The following are some advantages of using CHAPS in place of cholate in the reagent:

1. CHAPS is more soluble and also more easily reconstituted by plasma than is cholate, thus it can more readily enhance pancreatic esterase activity than cholate.
2. As a result of the increased pancreatic esterase activity there is more chromogen production and thus the resolution and dynamic range of the test is increased.
3. Since CHAPS is more readily resolubilized than cholate, the diagnostic test strips will have greater precision with CHAPS because it will be maximally and more uniformly distributed throughout the reaction membrane immediately after the addition of blood to the strip.
4. Because of its different properties, CHAPS was found to be more compatible with a larger number of different chromogenic systems, particularly the Trinder systems than was cholate. In solutions made with cholate, 4-aminoantipyrine could not remain in solution.
5. Since CHAPS is a more acidic surfactant than cholate, it is able to go into and remain in solution at a lower pH than can cholate. This allows for lowering the pH of its reagent from 6.8 to 6.0 and thus eliminating uric acid interference.

Use of MES buffer in cholesterol reagent

- When MES, or 2-[N-morpholino]ethanesulfonic acid, was used as a buffer in cholesterol reagent in place of citrate, it was found to:
1. help solubilize and stabilize (keep in solution) certain cholesterol esterases necessary for the optimal performance of the assay.
 2. Increase the amount of color generated per mg/dL of cholesterol thus improving the test's precision. This is probably the result of better performance by the esterase(s).

Substitution of Rhodasurf ON-870 for Triton X-100 in a strip based D-3-hydroxybutyric acid assay

When Rhodasurf ON-870 (a mixture of ethoxylated tridecanol, ethoxylated
5 tridecanol phosphate ester, and phosphoric acid), or a similarly formulated
surfactant, was used in place of Triton X-100 (t-Octyphenoxypolethoxyethanol),
or a similarly formulated surfactant, in a dry, strip based D-3-hydroxybutyric acid
assay the following benefits resulted:

More ready solubilization and hence reconstitution of the tetrazolium indicator
10 resulting in:

1. Increased precision due to more uniform dispersion of reagents after
reconstitution with whole blood;
2. More rapid kinetics, resulting in the average test time dropping from 120"
to 40"; and
- 15 3. Increased dynamic range; from 0-10 mg/dL to 0-60 mg/dL D-3-
hydroxybutyric acid.

Enhanced stability of the D-3-hydroxybutyrate dehydrogenase.

The first benefit, as well as the three numbered entries that it subsumes, is
probably applicable to a number of assays using tetrazolium salts as a
20 chromogen, especially in a dry format.

ANALYTES

A wide variety of analytes can be determined by using the disclosed
apparatus. Examples are given in tables I and II, infra.

Further, given the small size and robust nature of the reagent strips and instrumentation, analyses need not be limited to traditional clinical laboratory settings. The device of the present invention is also simple enough to be used by people with minimal or no chemical or medical technology training. This advantage allows use at home, or by mobile health care delivery people. Examples of this are diabetics who must monitor themselves for glucose and ketone bodies, patients on home dialysis who would benefit by monitoring of urea nitrogen and people endeavoring to lower their cholesterol levels.

Further, by combining several different reagents on a single support, a panel of tests may be done. Examples of this would be a liver panel consisting of ALT, AST, Alkaline Phosphates. A diabetic panel might consist of glucose, beta hydroxybutyrate and glycated hemoglobin. A coagulation panel might consist of Prothrombin time, ACTT and ACT.

15

FAMILIES OF ANALYTES BY STRUCTURE

Table 1

Family	Examples
Carbohydrate	Glucose, lactose, galactose
Nitrogen Moiety	Urea nitrogen, creatinine, uric acid
Lipid	Cholesterol, triglycerides, LDL, HDL
Enzyme	ALT, AST, Alkaline Phosphatase, CPK, CK-MB
Hormone	Hcg, lh
Therapeutic Drugs	Theophylline
Drugs of abuse	Cocaine, marijuana, barbiturates, salicylates
Electrolyte	Na + K + Cl + Li + CO ₂

Nucleic Acids	Infectious disease, forensic applications, genetic disorders
---------------	--

FAMILIES OF ANALYTES BY DISEASE

Table II

Disease	Examples
Diabetes	Glucose, beta hydroxybutyrate, hemoglobin A _{1c}
Liver problems	ALT, AST, bilirubin
Acidosis/Alkalosi	PO ₂ , PCO ₂ , pH
Hypertension	Na ⁺ , K ⁺
Nutritional status	Ca, Mg Zn trace minerals

5

Examples

The following illustrative examples teach various combinations of buffers, dyes, stabilizers and other reactive and functional components which may be combined by a person having ordinary skill in the art into the system test reaction areas.

10

Table IX gives various types of dyes and indicators used in diagnostic reagents.

Example #1

Glucose measuring system

15 Table III

Ingredient	Function	Amount	Available from:
Glucose Oxidase	Reactant	25,000	Sigma Chemicals

			St Louis, MO
Peroxidase	Reactant	75,000	Sigma Chemicals St. Louis, MO
Silwet 7500	Serfactant	0.10 ml	Dow-Corning Midland, MI
PVP K 30	Enzyme Stabilizer	0.50 gms	ISP Linden, JN
Citric Acid	Buffer System	1.25 gms	Aldrich Chemical Milwaukee, WI
Sodium citrate	Buffer System	0.10 ml	Dow-Corning Midland, MI
DOW 1520	Antifoam	1.00 gms	Aldrich Chemical Milwaukee, WI
4 AAP	Chromophore	0.25 gms	Aldrich Chemical Milwaukee, WI
3 5 DCHBS	Chromophore	0.25 mgs	Boehringer Mannheim
Distilled H ₂ O	Solvent	QS to 100 ml	

Preparation: Approximately 50 ml of distilled H₂O was placed in a beaker on a stirring plate. A magnetic bar was added and the ingredients added sequentially after the previous gradient was dissolved and dispersed. After all ingredients

- 5 were added the volume was adjusted to 100 ml of distilled H₂O .

Example #2

Triglycerides measuring system

TRIGLYCERIDES + H₂O ^{CHOLESTEROL ESTERASE} GLYCEROL + FREE FATTY
 ACIDS GLYCEROL + ATP ^{GLYCEROL KINASE} L – ALPHA – GLYCEROPHOSPHATE
 + H₂O₂ H₂O² + 4-AMINOANTIPYRINE + DCHBS ^{PEROXIDASE} QUINONEIMINE
 CHROMOPHORE

5 Table IV

Ingredient	Function	Amount	Available from
Cholesterol esterase	Reactant	15,000 units	Shinko-American NY, NY
Glycerol kinase	Reactant	5,000 units	Shinko-American NY, NY
Glycerophosphate Oxidase	Reactant	5,000 units	Shinko-American NY, NY
Peroxidase	Reactant	5,000 units	Shinko-American NY, NY
4 AAP	Chromogen	1.0 ogm	Aldrich
3, 5 DCHBS	Chromogen	0.25 gm	Boehringer Mannheim
MES	Buffer	2.50 gm	Research Organics
PVP K30	Stabilizer	0.50 gm	ISP
Glucose	Filler	2.50 gm	Sigma
Triton X-100	Surfacant	0.10 gm	Boehringer Mannheim
Distilled H ₂ O	Solvent	QS to 100 ml	

Preparation: Same as example #1

Example 3

Cholesterol measuring system (all amounts approximate)

Table V

Ingredient	Function	Amount	Available from
Choleseterol oxidase	Reactant	10,000	Shinko-American NY, NY
Cholesterol esterase	Reactant	7,000	Shinko-American NY, NY
Sodium phosphate 0.5 M pH 7.0	Buffer	750 ml	Dow-Corning
BSA	Surfacant	15 gm	Aldrich Chemical
Peroxidase	Reactant	170,000	Shinko-American NY, NY
DOSS	Surfactant	7.0 gms	Boehringer Mannheim
Sucrose	Stabilizer	1.0 gms	Sigma Chemicals
TMB	Chromogen	10.0 gms	Aldrich Chemical
Distilled H ₂ O	Solvent	QS to 100 ml	

5 Preparation: Same as example #1

Alternatively, the chromogen may be prepared in an organic solvent matrix and treated as a first or second application to the membrane or paper.

Table VI

Ingredient	Function	Amount	Available from
Acetone/methanol	Solvent	100 ml	Aldrich

1:1			
Tetramethyl benzidine	Solvent	1.0 ogm	Biosynth Inc Chicago, IL

Example 4

Blood Urea Nitrogen Measuring System

Table VII

Ingredient	Function
Urease	Reactant
H ₂ O	Solvent
Bcomthymol blue	Chromogen
PVP K90	Film former
Fructose	filler

5

Preparation: Same as experiment #1

Table VII*Types of Indicators*

Chromogenic substrate

- 10 Redox
- Leuco dyes
- Oxidative couplers
- Benzidene Derivatives
- Fluorescent labels
- 15 Dye releasing system

Table IX

Separation mechanisms used in dry reagents

Chemical	Physical	Mechanical
Dextran	Hydrophilic polymers	Centrifuge
Sugars	Porous latex films	Filters
Lectin	Polymer & swelling agent	Filters & pressure
Amino acids PEG/polyacrylate	Membranes microfiber cloth	Membranes & differential pressure wedge shape
Thrombin	Napped cloth	Gels
Sintered porous matrix	Coagulants	Density gradient
Agglutinating agents	Glass fibers	
Amine polymers	Hollow fibers	
Trivalent cations	membrane	

Example 5

5 HDL Formulation

Table X

Chemical	Quantity/100ml
DI WATER	80 g
PTA	1.70 g
Mg Sulfate	0.75 g
Sodium EDTA	0.10 g
DiPotassium Phosphate	1.21 g
QS to 100 ml	

10

Example 6

LDL Formulation

15

Micell layer (i.e, Michele layer) S&S 595 coated with Surfynol (.9%); Alpha-Cyclodextrin (1.5%) Tween 20 (0.05%); BSA (1%); NaCL (1%); d-Sorbitol (1%). Separation Layer, BTS#45, BTS#5, or Millipore PES tight side down, coated with Casein (Pierce Blocking Solution) and 1%D-Sorbitol. Standard Cholesterol

5 Membrane, Biodyne A.

SPECTROPHOTOMETER

The present invention also includes use of a spectrophotometric device 10
10 for determining the density of the color reaction on and in the membrane surface of the test reaction layer 32 within test strip 12. Photometric device 10 as shown in Fig. 1 includes a hand-held housing 50 for containing electronic control circuitry for operation the aforementioned tests. In the embodiment shown in Fig. 1, a test strip holding region 52 is located above three light detectors or sensors 54 each
15 disposed within a port 56. During test operation, a test strip 12 is inserted into holding region 52 so that test strip openings 26 are located adjacent ports 56. Light sensors may take a reading from light reflected from the exposed test reaction membrane layer 32 or from test strip 12 itself to determine its color.

Housing 50 further includes a specialized display device, such as a liquid
20 crystal display 58. Display 58 is utilized for relating test results and other information to the user. In particular, a color scale 60 is used to facilitate interpretation of test results operating concurrently with digital display segments 62. Additional display segments on display 58 include a test wait indicator segment 64 to inform the user to wait while device 10 is performing the selected

tests, and a test name segment 66 which the unit determined from the type of test strip 12 inserted.

Color scale 60 may easily be constructed by a plurality of shaded or colored segments arranged adjacent each other to form a bar graph like indicator. Electrically controllable segments 68 are oriented over the color or shaded segments so that when segments 68 are activated segments 68 become dark, preventing certain colored or shaded segments 60 from being visualized or viewed. Segments 68 that are not activated permit the underlying colored or shaded segments of color scale 60 to be visualized. In this way it is possible for an electronic control to permit only a single colored or shaded segment to be viewed thereby communicating test results.

A possible result range spectrum for color scale indication segments may include particular colors with particular test result meanings such as:

	Very high result danger, RED
15	High result danger, RED
	High result caution, YELLOW
	High result caution, YELLOW
	High normal result, GREEN
	Normal result, GREEN
20	Normal result, GREEN
	Low normal result, GREEN
	Low result caution, YELLOW

Low result caution, YELLOW

Very low result danger, RED

Color scale 60 permits an unsophisticated user to instantly visually

5 determine, in one embodiment, if a test result is normal (a green segment visualized), slightly abnormal (a yellow segment visualized) or dangerous high or low result (a red segment visualized). Alternatively, if a color liquid crystal display is utilized, the electronic control for test unit 10 may directly indicate a colored segment, rather than covering all but one colored segment.

10 A suitable instrument, such as a diffuse reflectance spectrophotometer 10 with appropriate software, can be made to automatically read reflectance at certain points in time, calculate the rate of reflectance change, and by using calibration factors and software, output the level of analyte in the fluid tested. The electronic control mechanism of photometric unit 10 is shown in schematic

15 form in Fig. 5. One or more light sources 70, for example high intensity light emitting diodes (LED) are disposed in housing 50 to illuminate test strip 12 as shown by arrows 72. A light detector or sensor 54 can be adapted to generate or respond to particular wavelengths of light.

Sensor 70 transmits a signal to an amplifier 74 as is known in the art. One

20 type of amplifier available for use is, for example, a linear integrated circuit, which converts the phototransistor current to a voltage signal.

Appropriate electronic circuitry is utilized to take the output of amplifier 74, normally a sample and hold unit 76, and transfer the signal to an analog-to-digital converter 78. Analog-to-digital converter takes the analog voltage output from the

25 sample and hold unit 76 and converts it to, for example, a 16 bit binary digital

number upon command of a microprocessor/microcontroller unit 80. Preferably an electronic microprocessor/microcontroller 80 utilizing digital integrated circuitry is used to time selected tests, read signals and together with associated programs and data memory 82, calculate and store reflectivity values and

5 calculate analyte levels from the stored data.

Additional information for particular tests may be stored in a removable EEPROM unit 84 operably connected to microprocessor/microcontroller 80. EEPROM unit 84 is an interchangeable plug-in memory module containing measurement parameters, software, calibration data, and reagent recognition

10 data for particular test strips 12. Additionally, EEPROM unit 84 contains the shelf life data and identity verification information for particular production runs or lots of test strips 12.

Automated lot coding is done by color coding the plastic material used to make the test strip holder 12. The color used in test strip holder 12 preferably

15 has 16 different densities that can be distinguished by at least one of the wavelengths used in the optical sensor head 54 of instrument 1.

As the strips 12 are inserted into device 10, the instrument detects a change in the measurement area. This change indicates that a strip 12 has been

20 inserted into the instrument 10. As the instrument detects the insertion of a test strip 12, it reads the densities of at least one of the LED's and calculates the lot number by the above table. Instrument 10 then goes to the EEPROM port connected to microprocessor/microcontroller 80 which has an EEPROM unit 84 inserted. Instrument 10 checks to see that the EEPROM preselected lot number

25 is the same as lot number of test strip 12 that had been inserted into the

instrument. If the lot numbers are the same for test strip 12 and EEPROM 84, the instrument downloads the information contained in the EEPROM and proceeds with the test analysis. The instrument 10 reads the density of the unreacted strip to assure quality of the strip before the test is initiated, if quality is
5 passed then the instrument instructs the user to apply a sample.

A sample is then applied and instrument 10 begins a measurement cycle to ensure that the proper amount of sample was applied to the test strip. When the instrument has determined that enough sample has been applied, it then goes into another cycle to measure the end of the chemistry reaction. When the
10 end of the chemistry reaction has occurred, then the instrument measures the final density and compares it to a measurement algorithm stored in EEPROM unit 84. This measurement algorithm then determines the concentration of the test to be measured by comparing the measured density (darkness) of the color formed and comparing this density number to a table of values through the use of an
15 algorithm stored in the EEPROM unit 84.

After a particular test strip is selected and placed in the unit, a sample normally a whole blood sample from a fingertip or from a pipiter tip (which could have gotten its sample from a tube of blood as in a laboratory type situation), is applied to the sample application spot, opening 24, on test strip 12. A
20 disbursement layer 28 causes the sample to quickly spread over the entire area of carrier layer 14. The separation layer 30 of the test strip spot is allowed to separate out the solids (red blood cells and other analytes disrupting elements) from the liquid (plasma or sera or other analyte containing portion). The separated fluid, i.e., the plasma, sera, or other analyte containing portions, moves
25 to the test reaction membrane layer 32 below the separation membrane 30. The

above fluid migration causes the reactants (analytes such as glucose) in the sample to come into contact with the reactants in test reaction membrane layer 32.

Analytes/fluid contacts reagent layer reaction 32 and initiates an appearance or disappearance of color, depending on its particular reaction. The above presentation of analyte to the reaction layer 32 causes the desired reaction to occur. This reaction causes a color change that can be detected both visually and by the instrument. The color change is then converted into a digital result on the instrument LCD as described above. A comparison color chart can be used to visually determine a reaction quantity scale as in litmus paper.

Instrument 10 can use different wavelengths at different density portions of the reaction to maximize the dynamic range of the chemistry and the limits of the instrument at a particular wavelength.

The "end-point" of the reaction is defined as a point where there appears to be no change or a very small change in density. That is, the chemistry changes color proportional to the concentration of the reactance that has come into contact with the reactance materials in the test pad (membrane). This small amount of change can be a change per time period. An example would be as per the graph in Fig. 6. Detailed information used to generate this graph is that the changes per 5-second time period during the beginning of the test reaction would be greater than 5% reflectance per 5-second time period. When this change is less than 1% reflectance per time period it can be said that the reaction is complete or at an endpoint. The instrument stores this percentage reflectance at this time and uses it as above to determine the concentration of the analyte tested for in the test strip.

The Kubelka-Monk equation of $K/S = (1 - \text{reflectance})^2$ divided by $(2 \times \text{reflectance})$ can be used to linearize the percentage reflectance values. This linearization simplifies the algorithm necessary to calculate results. This pseudo endpoint chemistry allows a more stable read time, which in turn allows for a more reproducible answer. Pseudo endpoints also permit a more rapid assay to be performed. Certain other glucose monitoring systems incorporate pre-determined timing circuit. This pseudo endpoint allows for a different method to be used in measuring chemistry reactions, provided one can determine the endpoint of the chemistry by a method other than timing.

Multiple wavelengths are used to enhance the dynamic range of chemistry. This is particularly useful when one uses a multiple chromophore indicator system as do some of the above mentioned chemistries. Early portions or low concentrations of a test such as glucose can use a broad range indicator such as TMB to increase sensitivity in the low to mid range of the chemistry. When the test concentration is higher or the reaction faster, a different chromophore is focused upon to determine more dynamic range than the previous chromophore. This allows one to expand the dynamic range by two different methods.

One can also use wavelengths on the peak for more dynamic range and wavelengths off the "peak" absorbance of the test system to enhance or reduce dynamic range and also to enhance or reduce the "pseudo endpoint" algorithms. Manipulation of these four factors, chromophore A, chromophore B, wavelength 1 and wavelength 2 can allow one to better define the "pseudo endpoint" algorithm and also allow one to optimize the dynamic range of the chemistry which in turn

allows for increased sensitivity throughout the chemistry reaction range with greater precision.

Multiple wavelengths can also be used with different angles of emission of correct possible problems in positioning the strip in the instrument. If the detector is a "0"

- 5 angle and the emitters of the same or different wavelengths are at different angles (one at 40° and one at 50°) the tilting of a surface will positively contribute to one reading while the other contributes in a negative manner, thus canceling the error presented by the angle presentation of the surface. These same measurement methods can be used to eliminate interference from substances
- 10 such as bilirubin and others. When the angle of light incidence is increased from improper positioning of a chemistry read surface to the instrument optics, errors of both gloss and angularity are introduced into the measuring system and can give false low readings.

15 Examples

1. Indicators and chromogens advantageously used in combination

a. wide range pH test

Bromothymol blue and methyl red covers pH range of 5 through 9

b. 4 amino antipyrine + 3.5 dichlorohydroxybenzene sulfonate (4AAP _3.5
20 DCHBS)

c. TMB + Chromotropic acid

d. Syringaldazine _ Vanillin Azine

2. Color coding for test and lot identification

a. Blues, 16 different shades (density)

25 b. Reds, 16 different shades (density)

- c. Greens, 16 different shades (density)
- d. Yellows, 16 different shades (density)
- e. Oranges, 16 different shades (density)
- f. Browns, 16 different shades (density)
- 5 g. Magentas, 16 different shades (density)
- h. Light blues, 16 different shades (density)
- i. Light reds, 16 different shades (density)
- j. Light greens, 16 different shades (density)
- k. Light browns, 16 different shades (density)
- 10 l. Light magentas, 16 different shades (density)
- m. Cyan, 16 different shades (density)
- n. Light cyan, 16 different shades (density)

It will be appreciated that the foregoing is presented by way of illustration only,
15 and not by way of any limitation, and that various alternatives and modifications
may be made to the illustrated embodiment without departing from the spirit and
scope of the invention.

What is claimed is:

1. A process for measuring cholesterol in low density lipoproteins present in a living sample by optically measuring a reaction product of the living sample with a reagent, which comprises conducting the reaction of the living sample in the presence of an non-ionic surfactant and at least one member selected from the group consisting of cyclodextrin and derivatives thereof.
2. The process according to claim 1, wherein the cyclodextrin derivatives is at least one compound selected from the group consisting of dimethyl-alpha-cyclodextrin and poly-beta-cyclodextrin.
3. The process according to claim 1, wherein the cyclodextrin derivatives is poly-beta-cyclodextrin.
4. The process according to claim 1, wherein the amphoteric surfactant is at least one compound selected from the group consisting of alkyl betaine derivatives, imidazolinium betaine derivatives, sulfobetaine derivatives, aminocarboxylic acid derivatives, imidazonline derivatives, amine oxide and ethoxylated acetylene derivatives.
5. The process according to claim 1, wherein the amphoteric surfactant is at least one compound selected from the group consisting of an aminocarboxylic acid derivative, lauric acid amidopropyl betaine, a 2-alkyl-N-carboxymethyl-N-hydroxyethyl imidazolinium betaine lauryl betaine, sodium N-lauroyl-N-methyl-beta-alanine and N-octyl-N, N-dimethyl-3-amminio-1 propanesulfonic acid.
6. A process for measuring cholesterol in low density lipoproteins in a living sample, which comprises: treating the living sample with a first reagent comprising at least one member selected from the group consisting of

cyclodextrin and derivatives thereof along with a suitable surfactant;
measuring reflectance resulting in color on a membrane reactive to
cholesterol; containing cholesterol oxidase, cholesterol esterase, and
peroxidase with electron acceptors which change colors; and providing the
5 cholesterol amount in the living sample on the basis of the reflectance data
measured above, wherein a coupler, a developer, peroxidase, a surfactant
and cholesterol esterase are contained in at least one or two layers.

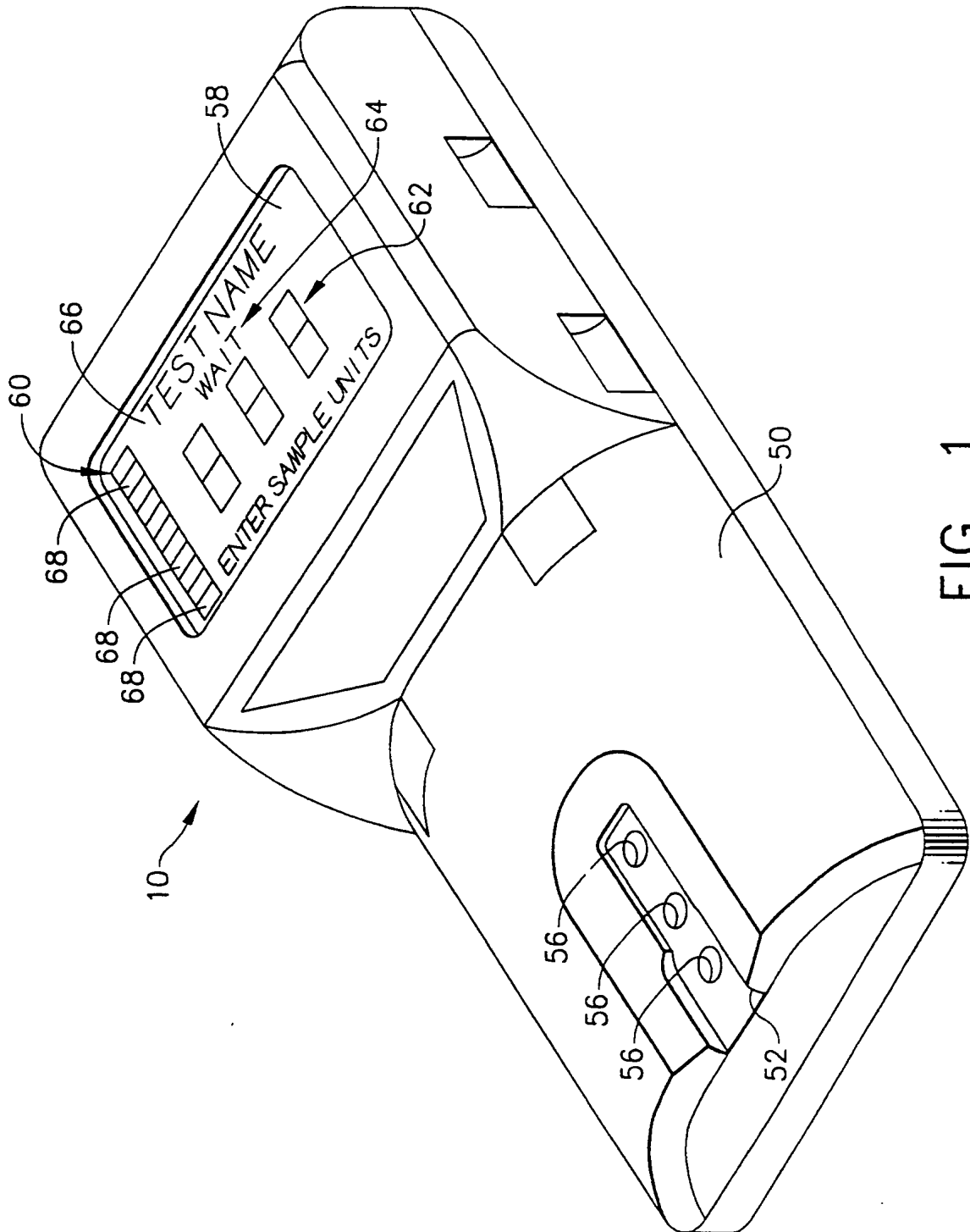


FIG. 1

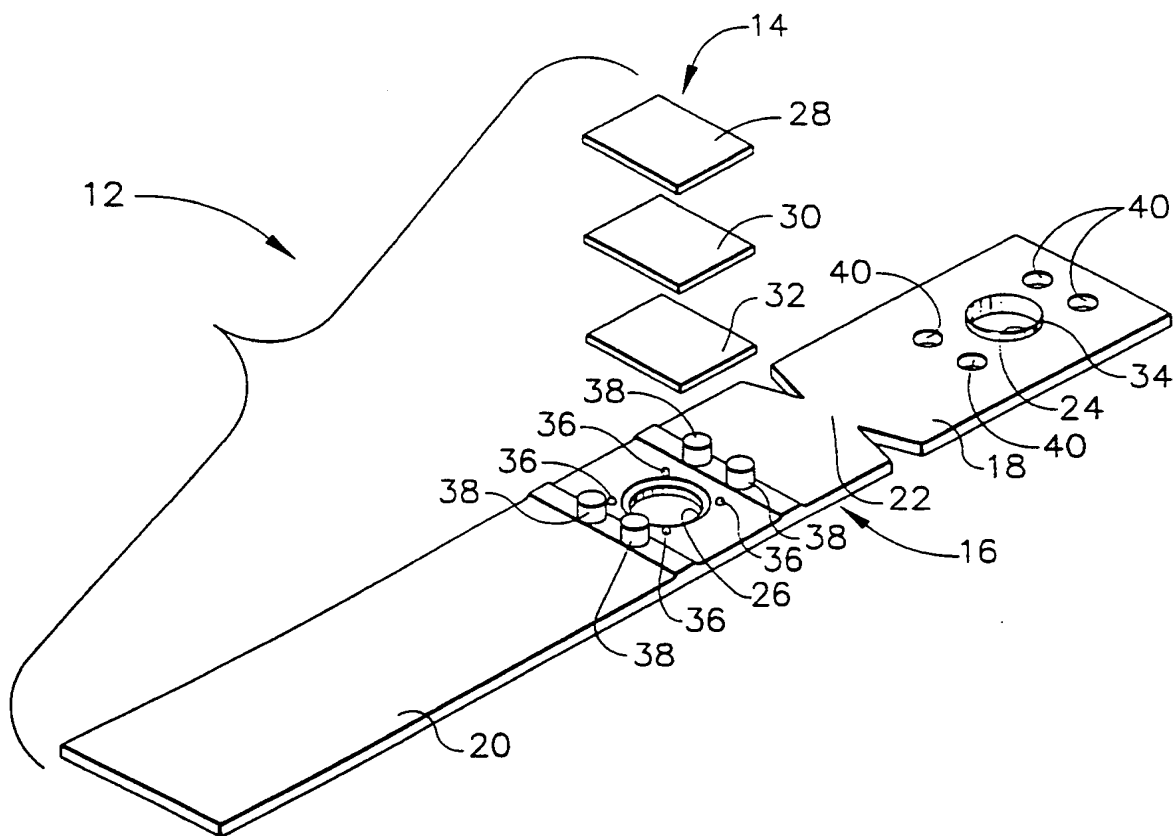


FIG. 2

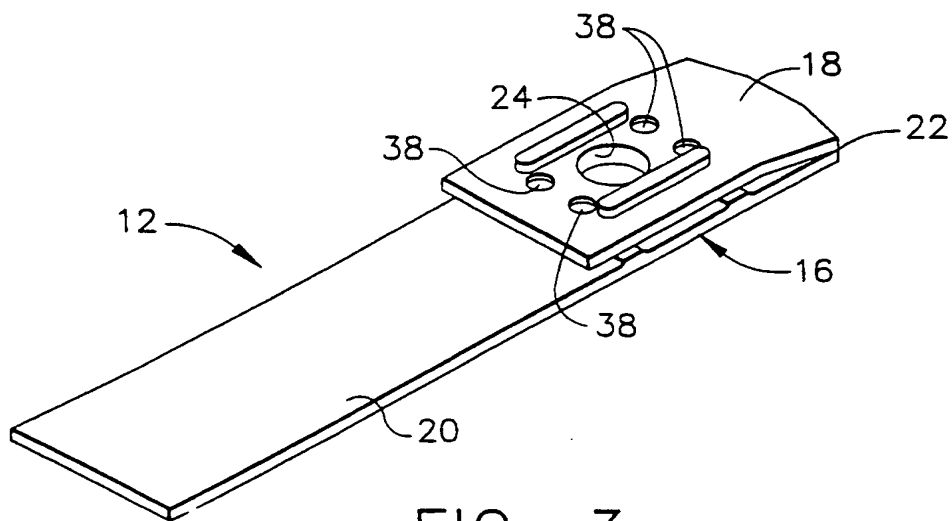


FIG. 3

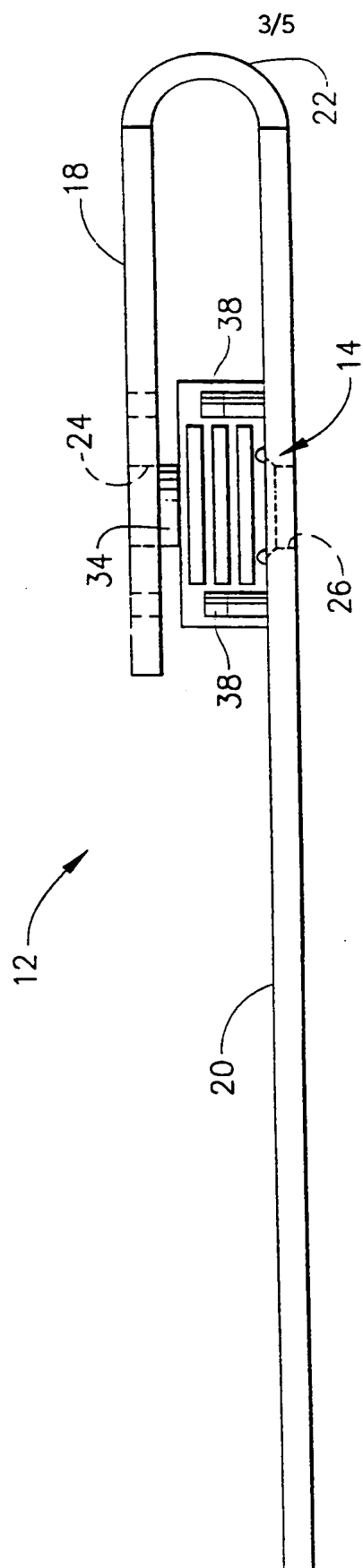


FIG. 4

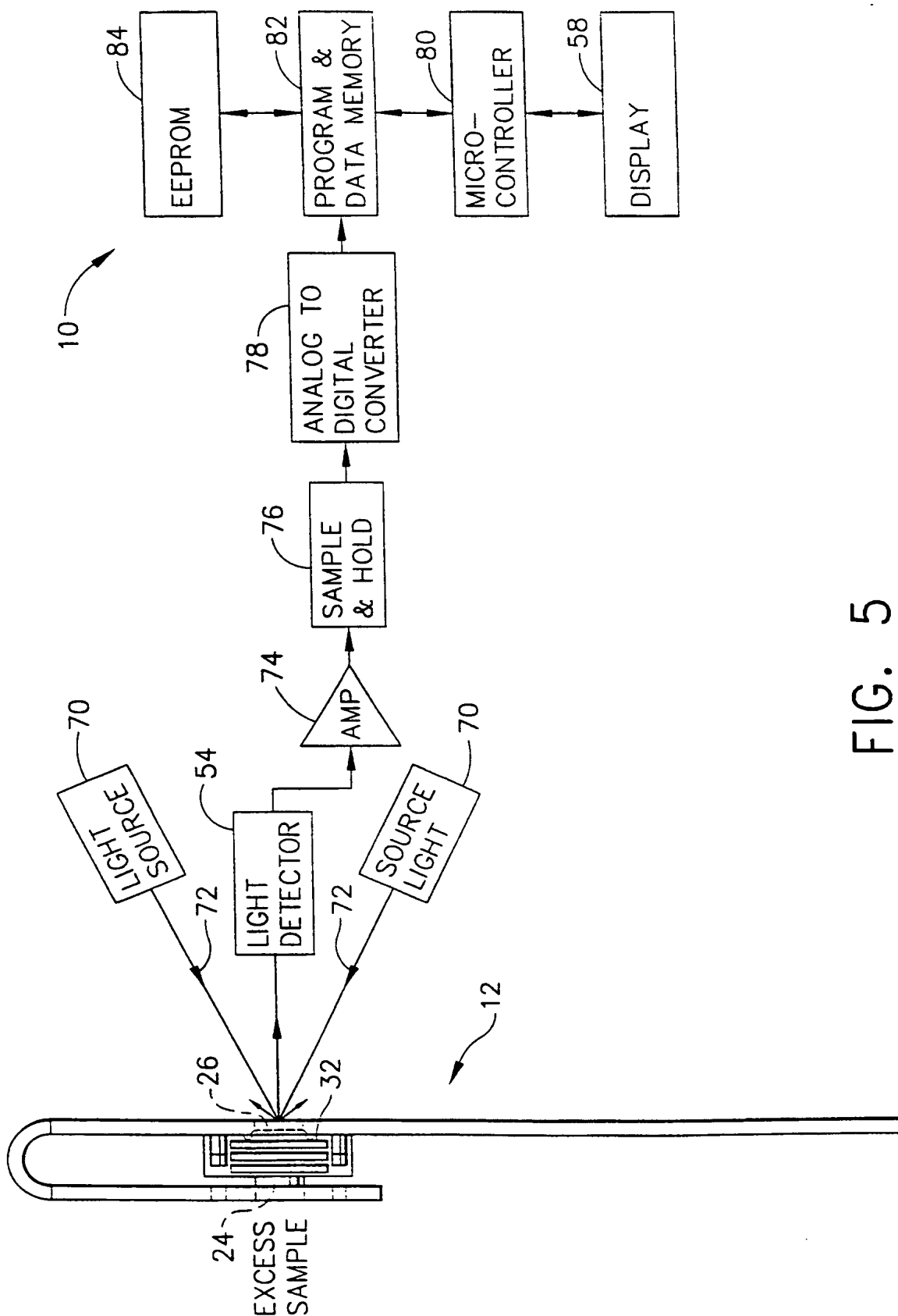


FIG. 5

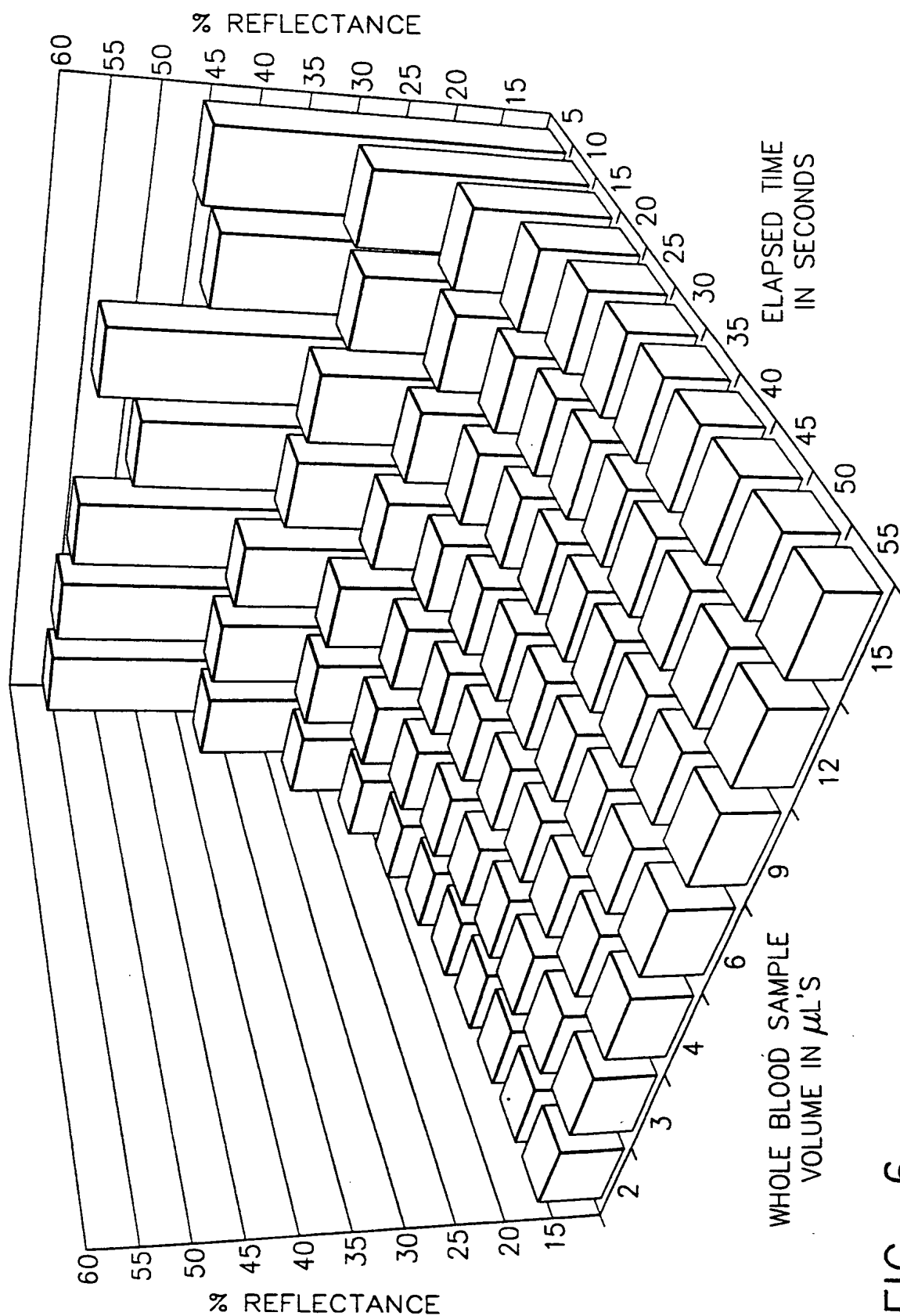


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/16816

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/44, 1/60, 1/26, 1/28, 00; C08B 37/16

US CL : 435/19, 11, 25, 28, 5; 536/103

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/11, 25, 28, 5; 536/103

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS: cholesterol measurement in LDL, non-ionic surfactant, cyclodextrin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,814,472 A (MIKI et al) 29 September 1998 (29.09.98), see abstract, col. 2, lines 15-68, col. 3, lines 1-16, col. 5, lines 35-65.	1-6
X	US 5,879,901 A (FUTATSUGI et al) 09 March 1999 (09.03.99), col. 3, lines 6-58 and col. 9, lines 9-29.	1-6
X	US 5,807,696 A (MIYAUCHI et al) 15 September 1998 (15.09.98), abstract, col. 1, lines 5-10, col. 6, lines 12-68.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 SEPTEMBER 2000

Date of mailing of the international search report

13 OCT 2000

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